Imaging-free, few-shot, three-dimensional focusing on point-like emitters in confocal microscopy

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Abstract: We introduce a rapid, noise-robust, three-dimensional focusing framework for as-is confocal microscopes. We show automated real-time focusing on nanoscale emitters for SNR down to 1, and position tracking with a precision below 10 nm.

Introduction: Confocal microscopes are widely employed to optically study individual fluorescent point-like emitters (PLEs). Co-locating the focal point of the microscope objective with the position of the PLE under observation, which we define as focusing, is essential to collect a near-maximum in the detected fluorescence intensity. Rapid, 3D focusing with sub-100 nm precision is critical to analyze the properties of PLEs or PLE-tagged objects on a large scale [1]–[3]. Conventionally, confocal microscopes focus on PLEs by iteratively adjusting the axial z-focusing to position the PLE in the focal plane, coupled with x-y imaging through raster scanning [2]. Such methods are often slow, increase susceptibility to phototoxicity and photobleaching, and may necessitate the use of additional optical components [4]. Here, we introduce FiND, Focusing in Noisy Domain, a rapid, real-time, and noise-robust 3D focusing framework compatible with standard confocal microscopes, requiring no training, imaging, or hardware modifications [5].

Methods and Results: To model focusing, we assume that the excitation beam's focal point has a coordinate **r** referenced to the PLE position. Within an iterative algorithm, the focal point moves under the influence of the ground truth and noise information received from local intensity measurements (Fig. 1(a)). We model the photon detector signal, $s = e^{-\frac{r^2}{2}} + n$, as a sum of a symmetric Gaussian point spread function (PSF) and a uniform Gaussian noise (standard deviation $\bar{n}$=1/SNR). The end goal is to move the focal point into a target zone, close to the PLE, and maintain it there. In this case, the target zone is defined as a sphere of radius $r_\varepsilon = 0.44$ around the PLE. The average collected intensities in this target zone are above $1 - \epsilon = 0.9$, where $\epsilon = 0.1$ is a predefined tolerance. A focusing attempt is deemed successful if the collected intensities remain above 0.9 on average upon entering the target zone. Focusing time, t_{focus}, is the iteration upon which the focal point first enters the target zone in a successful focusing attempt.

The idea of FiND is to find optimal sampling parameters that will allow successful focusing in the shortest t_{focus}. We develop the framework by modeling the ground truth information as an attractive force towards the PLE, while the noise is represented as a repulsive force away from the PLE, along a single dimension. Here, we illustrate this concept with the example of a simple finite difference sampling scheme. Specifically, we assume that in one iteration, the signal $s(\mathbf{r},t)$ is sampled at six points located a step size of δ away from the current position **r** (Fig. 1(b)). After sampling, the focal point is displaced by $\mathbf{D} = \frac{\lambda}{2\delta}\sum_{j=x,y,z}\{s(\mathbf{r}+\delta * \mathbf{e}_j) - s(\mathbf{r}-\delta * \mathbf{e}_j)\}\mathbf{e}_j$ where λ is the learning rate and $\mathbf{e}_j$ are unit vectors. **D** is the sum of the ground truth displacement $\mathbf{D}_{\mathbf{GT}}$ and the noise displacement $\mathbf{D}_{\mathbf{N}}$. While the displacements add up vectorially in three dimensions, the contributions of these components to the effective radial coordinate r^2 add up algebraically. This allows us to examine them intuitively as forces acting on a particle along the single coordinate r^2 (Fig. 1(b)). The resultant force is $F_{res} = -2r\frac{\lambda}{\delta}e^{-\frac{r^2}{2}-\frac{\delta^2}{2}}\sinh(r\delta) + \frac{\lambda^2}{\delta^2}e^{-r^2-\delta^2}\sinh^2(r\delta) + \frac{3\lambda^2\bar{n}^2}{2\delta^2}$, which needs to be attractive for successful focusing. This expression is validated by good quantitative agreement with Monte-Carlo simulations. We select sampling parameters λ and δ for rapid focusing by maximizing $-F_{res}(r_\varepsilon)$ at the target zone boundary.

To experimentally benchmark the performance of FiND, we repeatedly focus a standard confocal microscope on a subwavelength-sized nanodiamond containing fluorescing nitrogen-vacancy centers (NV-ND). For each attempt, we record the focusing time and evaluate how t_{focus} scales as a function of SNR (Fig. 1(c)) and the starting position $\mathbf{r}_0$ (Fig. 1(d)) normalized by the microscope's PSF width (~100 nm). We find good agreement between the experimental t_{focus} values and t_{focus} predicted by analytical theory and Monte-Carlo simulations. Additionally, we observe few-shot focusing for SNRs > 5 and $|\mathbf{r}_0| < 2$, and consistently successful focusing for SNRs down to 1 and $|\mathbf{r}_0|$ as high as 4.

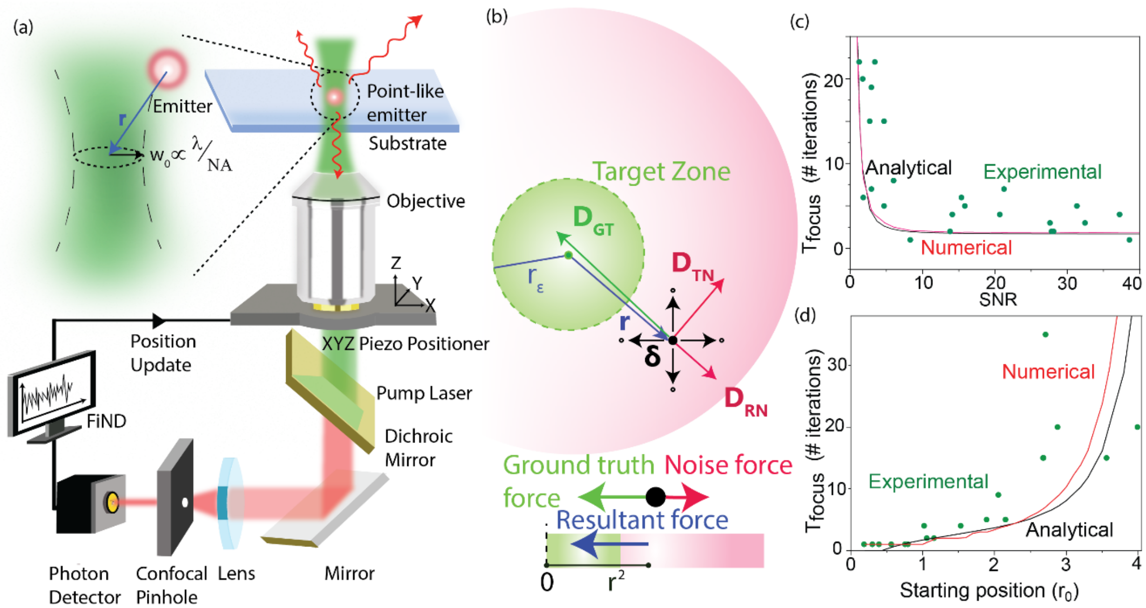

Fig. 1: (a) A standard confocal microscope setup for rapid 3D focusing on PLEs using FiND. (b) Schematic of displacements in real space and forces in the 1D space in FiND framework. (c) Scaling of focusing time as a function of SNR for constant $r_0 = 1$. (d) Scaling of focusing time as a function of the starting position at constant SNR = 26.

Furthermore, the sharp PSF of the confocal microscope can be harnessed for 3D tracking of individual fluorescent nanoparticles over large spatial and temporal ranges [6]. By running the focusing algorithm continuously on a freely drifting microscope, and recording the objective coordinates, we observe that the 3D position of a NV-ND can be tracked indefinitely. We estimate the positional uncertainties of the PLE location by analyzing nearly constant segments in the coordinate traces obtained from the piezo nanopositioning stage. We find uncertainties of $\Delta_x \sim 9$ nm, $\Delta_y \sim 8$ nm, $\Delta_z \sim 9$ nm and are limited by the coordinate read-out noise of the piezo stage.

FiND predicts optimal focusing parameters through a simple analytical model, enabling rapid, noise-robust few-shot focusing at low SNRs. With the finite difference scheme, FiND outperforms focusing based on natural evolution strategy (NES), particle swarm optimization (PSO), and CNN-based curve fitting in terms of noise resilience and focusing speed. It enables large-scale automated PLE characterization [2], [3], indefinite real-time 3D tracking (<10 nm precision), drift correction, and microscope stabilization. The memoryless nature of FiND's iterative sampling method allows one to focus even on blinking emitters, useful for super-resolution microscopy techniques that leverage this property [7].

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